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INTRACELLULAR pH REGULATION IN CULTURED MOUSE OLIGODENDROCYTES

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SUMMARY

1. Intracellular pH (pH_i) and the mechanism of pH_i regulation have been investigated in cultured oligodendrocytes from mouse spinal cord using double-barrelled neutral-carrier H^+-selective microelectrodes. The distribution of H^+ was not in electrochemical equilibrium. The pH_i was more alkaline than the pH of the bathing medium (pH_o), namely 7·5 at pH_o 7·2 and 7·6 at pH_o 7·4.

2. Removal of HCO_3^- from the bathing medium reduced the steady-state pH_i by 0·4 units. An increase in extracellular K^+ caused, with a delay, an increase in pH_i. A decrease in pH_o to 6·2 caused an acidification of pH_i by 0·5 units.

3. The pH_i regulation was studied by applying and subsequently removing NH_4^+ which resulted in an acidification of the cell. The subsequent recovery of pH_i could then be analysed. The recovery from an acidification by 1 pH unit lasted 3–10 min. In HCO_3^--free solution pH_i recovery was slowed.

4. In HCO_3^--free solution pH_i recovery was completely blocked when either Na^+ was removed or when amiloride was applied indicating an exclusive activation of the Na^+–H^+ exchanger.

5. In the presence of HCO_3^-, removal of Na^+ also completely blocked pH_i recovery. When Na^+ was readded, pH_i recovered. In HCO_3^--containing solution amiloride slightly slowed, but did not block pH_i recovery.

6. Removal of Cl^- or application of SITS, DIDS or furosemide, blockers of Cl^--coupled transport mechanisms, did not affect the pH_i recovery in the presence of HCO_3^-.

7. In conclusion, oligodendrocytes possess two mechanisms regulating pH_i, a Na^+–H^+ exchanger and a Na^+–HCO_3^- co-transporter while the latter is clearly more potent. It follows that pH_i regulation of oligodendrocytes is dependent on the transmembrane Na^+ gradient and is strictly separated from regulation of internal Cl^-.

INTRODUCTION

Interaction between neurones and glial cells is important in the function of the nervous system. Communication between these different types of cells is indirectly mediated via the extracellular space. One of the best, and most extensively described

examples of neurone–glia interaction is the regulation of extracellular K^+ by the glial syncytium thereby influencing neuronal excitability (Orkand, 1977; Coles & Tsacopoulos, 1981). But K^+ is not the only ion in the extracellular space which undergoes changes during neuronal activity. The pH in the interstitial space (pH_o) decreases during neuronal activity, often after an alkaline shift (Urbanics, Leniger-Follert & Lübbers, 1978; Kraig, Ferreira-Filho & Nicholson, 1983). These changes are moderate, but during intense stimulation of afferent pathways and direct stimulation of the nervous tissue, the pH_o falls by 0·2 units (Kraig *et al.* 1983). More dramatic changes in pH_o have been observed in pathological situations. During anoxia the pH_o has been found to decline consistently (Crowell & Kaufmann, 1961; Kraig *et al.* 1983; Mutch & Hansen, 1984). The magnitude of the pH_o change in complete ischaemia is usually 0·5 units, but can be significantly larger in animals rendered hyperglycaemic before the ischaemic challenge (cf. Hansen, 1985).

Changes of pH_o alter intracellular pH_i, both in neurones and glial cells (Moser, 1985; Chesler, 1986; Deitmer & Schlue, 1987). In turn, pH_i changes modify various ionic conductances in excitable cells, and the electrical coupling between adjacent cells is blocked by decreased pH (see reviews by Roos & Boron, 1981; Moody, 1984). In addition to its effects on other ionic conductances, H^+ might itself act as a charge carrier through H^+-specific channels in excitable cells (Thomas & Meech, 1982; Meech & Thomas, 1987). The pH_i is involved in a variety of other cellular functions (cf. Nuccitelli & Deamer, 1982). The late events of fertilization in sea urchin eggs are triggered by pH changes (Shen & Steinhardt, 1978; Gillies & Deamer, 1979), and protein synthesis rate is controlled in a reversible manner by pH_i (Grainger, Winkler, Shen & Steinhardt, 1979). It is also possible that changes in pH_o which result from nervous activity could function as a signal between neurones and glial cells. To detect such interactions, the carriers involved in the regulation of intra- and extracellular pH must be fully understood. The application of the technique of ion-selective microelectrodes has permitted characterization of pH_i regulation in neurones from lamprey, snail and leech (Thomas, 1984; Schlue & Thomas, 1985; Chesler, 1986) and glial cells from the leech (Deitmer & Schlue, 1987). The results indicate that Na^+–H^+ exchange across the cell membrane is one important pH_i regulating system in both glial cells and neurones. In the presence of bicarbonate, Cl^-–HCO_3^- exchange and in addition Na^+–HCO_3^- co-transport participate in glial pH_i regulation (Deitmer & Schlue, 1987). Indirect evidence for the existence of Cl^-–HCO_3^- exchange has been obtained for cultured primary astrocytes (Kimelberg, Biddlecome & Bourke, 1979) and LRM55 glioma cells originally isolated from a rat spinal tumour (Wolpaw & Martin, 1984). In both preparations the uptake and efflux of Cl^- ions were inhibited by the anion-exchange inhibitor SITS.

In the present study we have investigated pH_i regulation in cultured oligodendrocytes from embryonic mouse spinal cord to measure steady-state pH_i, to characterize the mechanisms involved in pH_i regulation and to better understand the influence of glial cells on the pH in the extracellular environment of the brain.

METHODS

General

Explant cultures were obtained from 13-day-old mouse embryos of NMRI mice. The spinal cord was dissected out under sterile conditions, cut into transverse slices and placed on glass cover-slips

coated with dry collagen (Sigma, Munich, F.R.G.). The explants were maintained without antibiotics in Eagle's Basal Medium with Earle's salts supplemented with 10% calf serum (Seromed, Munich, F.R.G.) at 35·5 °C in a humidified 3·5% CO_2–air atmosphere. Cultures were studied 3–6 weeks after dissection. Cultures contained astrocytes, oligodendrocytes, macrophages, fibroblasts and few neurones (Kettenmann, Orkand & Schachner, 1983*a*). Oligodendrocytes were identified by morphological criteria established through the use of cell-type specific antibodies O1 and O10 (Kettenmann, Sonnhof & Schachner, 1983*b*; Kettenmann, Sommer & Schachner, 1985). Cells were maintained on the stage of an inverted microscope and penetrated under visual control with the aid of a step-motor-driven micromanipulator (Sonnhof, Förderer, Schneider & Kettenmann, 1982).

Solutions

Cultures were continuously perfused with a bathing solution containing (in mM): KCl, 5·4; NaCl, 106·0; $NaH_2PO_4.H_2O$, 1·0; $MgSO_4$, 0·8; $CaCl_2$, 1·8; $NaHCO_3$, 26·2; HEPES, 10·0. NaOH was added to adjust the pH to 7·2 or 7·4. For HCO_3^--free solution $NaHCO_3$ was replaced by an equimolar amount of NaCl. For application of NH_4^+ 20 mM–NaCl was replaced by NH_4Cl. Na^+-free solution was obtained by replacing NaCl by choline chloride or *N*-methyl-D-glucamine, Cl^--free solution by replacing NaCl, KCl, $CaCl_2$ by sodium isethionate, potassium acetate, and calcium acetate, respectively. Na^+-free, HCO_3^--containing solution was obtained by perfusing Na^+-free solution with 2% CO_2 (and 98% O_2) for 2 h. Amiloride (2×10^{-3} M), 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS; 10^{-3} M), furosemide (10^{-3} M) and 4-acetamido-4′-isothiocyanatosilbene-2,2′-disulphonic acid (SITS; 10^{-3} M) were obtained from Sigma, Deisenhofen, F.R.G. and added to the bathing solution immediately before use. An increase in $[K^+]$ to 10 or 50 mM was achieved by replacing 4·6 or 44·6 mM-NaCl by KCl. External pH was reduced to 6·2 by using piperazine-*N*,*N*′-bis(2-ethanesulphonic acid) (PIPES) as buffer and adjusting the pH with HCl.

Microelectrodes

H^+-selective microelectrodes were made from theta-glass obtained from the university's workshop and with resins from Fluka, Neu-Ulm, F.R.G. (Schlue & Thomas, 1985; Kettenmann *et al.* 1983*b*). Electrodes had a tip size < 1 μm and their electrical resistance was 25–40 MΩ when filled with 2 M-potassium acetate. Silanization was accomplished by injection of vaporized trimethylchlorsilan (Merck, Darmstadt, F.R.G.). The reference barrel of the electrode was filled with 2 M-potassium acetate, and the pH-sensitive barrel with the resin superimposed with a chloride-containing citrate buffer, pH 6, saturated with 100% CO_2. Electrodes were calibrated prior to and after each measurement by changing the bathing solution from pH 7·2 to 6·2. The slope of the electrodes was in the range of 40–50 mV/decade of pH change. Electrodes with higher resistances of the reference barrel (> 35 MΩ) were less sensitive to changes in pH. We therefore had to find a compromise between a reasonable slope and a tip size which permitted stable penetration of cells. We commonly used electrodes with a resistance of 30 MΩ of the reference barrel measured in physiological saline. Ninety per cent of the response was typically reached within 5–20 s. Silver wires coated with AgCl served to connect the electrode-filling solutions with the head stages of the amplifiers. The reference electrode consisted of a Ag–AgCl pellet (WPI, New Haven, CT, U.S.A.) connected to the bath with a strip of filter paper.

Electronics and data storage

The wires from the ion-selective microelectrode were connected to a dual-stage amplifier (Meier, Munich, F.R.G.). The difference signals between the membrane potential and ion-selective channel and the membrane potential signals were digitized by an A/D converter, processed by microcomputer (IBS, Bielefeld, F.R.G.) and stored on a hard disc (Cameo, Eberfing, F.R.G.). A self-developed program package written in PASCAL permitted us to differentiate, display and plot stored data. To obtain the time derivative of the pH signal the difference between sample points of the voltage signal values was plotted against time. The sampling interval could be varied and was optimized to resolve the time course of pH_i recovery.

RESULTS

Resting pH_i

The steady-state pH_i was measured after penetration and before withdrawal of the H^+-selective electrode from cells when the membrane potential was stable for more than 15 min and > -50 mV. After penetration of a single oligodendrocyte, the pH_i was stable within 2 min; the membrane potential usually stabilized after

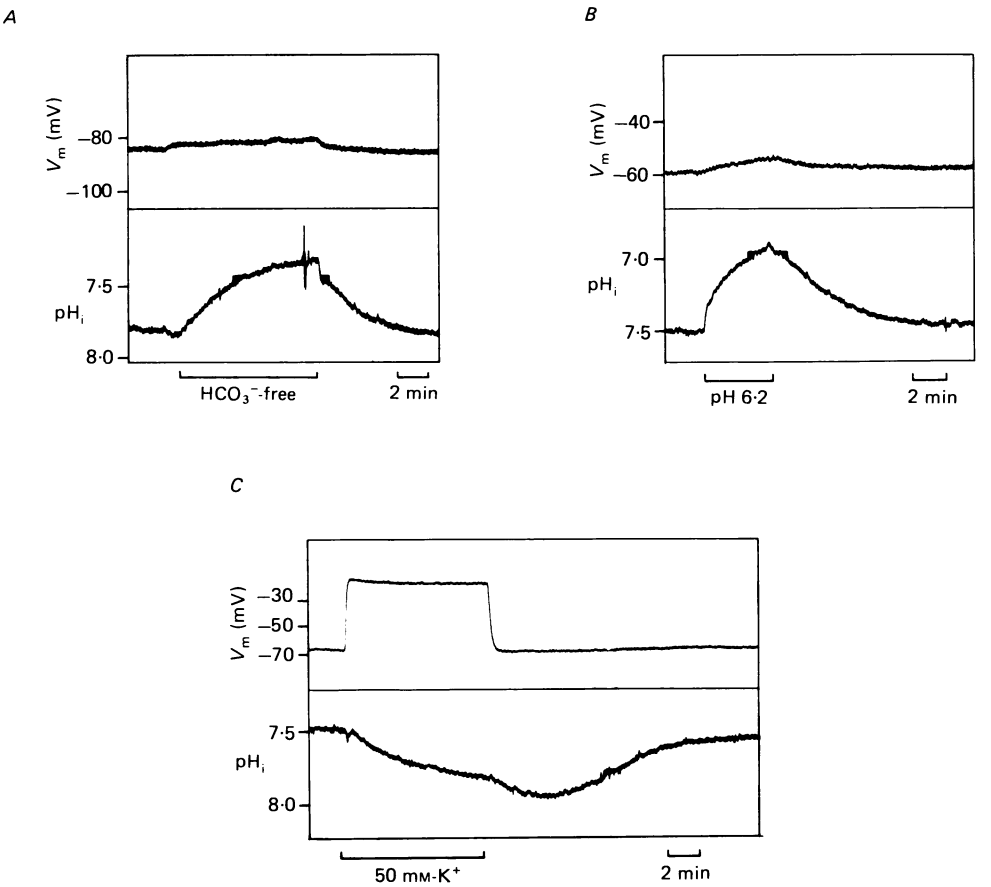

Fig. 1. Effects of changes in $[HCO_3^-]$, $[K^+]_o$ and pH_o on steady-state pH_i. *A*, HCO_3^- was changed from 26 mM to nominally HCO_3^--free solution as indicated by bar during recording of membrane potential (V_m) and pH_i. *B*, extracellular pH was decreased from 7·2 to 6·2 as indicated by bar. *C*, extracellular K^+ concentration was increased from 5·4 to 50 mM as indicated by bar.

5–15 min. The pH_i in the physiological bathing solution buffered at a pH_o of 7·2 or 7·4 was $7·5 \pm 0·15$ (mean ± S.D.; range 7·2–7·8, $n = 15$) and $7·6 \pm 0·2$ (range 7·2–7·9, $n = 16$), respectively. The membrane potential in these cells averaged -61 ± 6 mV (range -50 to -75 mV, $n = 31$). The difference between membrane potential and H^+ equilibrium potential at an extracellular pH of 7·2 or 7·4 was 81 ± 11 mV (range 60–98 mV, $n = 15$) and 71 mV ± 14 mV (range 46–90 mV, $n = 16$), respectively.

Transition from normal bathing solution to HCO_3^--free solution resulted in a decrease of pH_i by $0{\cdot}4 \pm 0{\cdot}15$ units (range 0·1–0·55, $n = 10$; Fig. 1*A*). In most, but not all cases, a transient alkaline shift lasting 1–2 min could be observed when HCO_3^- was removed (Fig. 1*A*). In these cells, addition of HCO_3^- resulted in a transient acidification. Subsequently the new steady state of pH_i was reached within about 5 min.

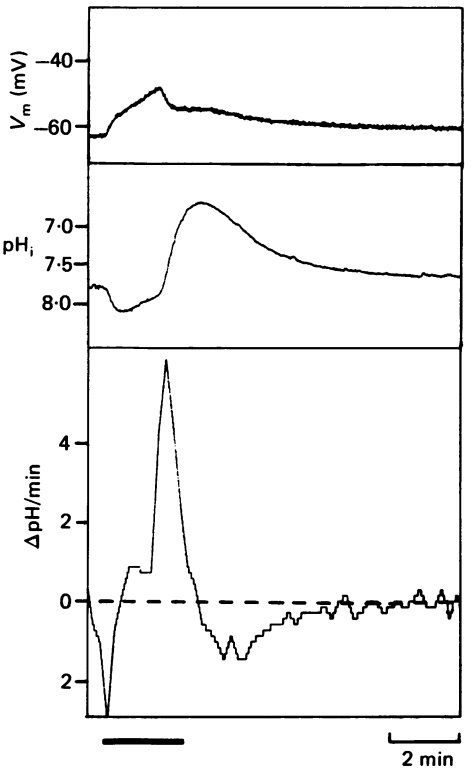

Fig. 2. Recovery of pH_i from acidification. During recording of membrane potential (V_m, upper panel) and intracellular pH (pH_i, middle panel) by an ion-selective, double-barrelled microelectrode, an oligodendrocyte was exposed to 10 mM-NH_4^+ as indicated by bar resulting in a depolarization of the membrane potential and an alkalization of the cell interior. The bathing solution contained HCO_3^-. Removal of NH_4^+ led to an acidification with a subsequent recovery. The trace of pH recording was differentiated by computer yielding the rate of pH change per time (ΔpH/min, lower panel). The large transients reflect the changes induced by application and removal of NH_4^+. During the recovery from acidification pH changes by one unit per minute.

Effect of changes in pH_o and $[K^+]_o$ on resting pH_i

The effect of changes in external pH and $[K^+]$ was tested on the steady-state pH_i of oligodendrocytes. Changes in external pH affected pH_i. When pH_o was lowered from 7·2 to 6·2, the pH_i slowly decreased by about 0·5 pH units (Fig. 1*B*). An increase of $[K^+]_o$ from 5·4 to 10 or 50 mM caused a slight increase in pH_i. After the re-addition of normal $[K^+]_o$ pH_i further increased by approximately 0·1–0·2 pH units before returning to resting level (Fig. 1*C*). These results demonstrate that

changes in the ion content of the external medium can affect the pH_i of oligodendrocytes.

pH_i recovery after acidification

We have used the NH_4Cl pre-pulse technique to acidify the cell interior and to study the subsequent recovery of pH_i (Boron & De Weer, 1976). After the application

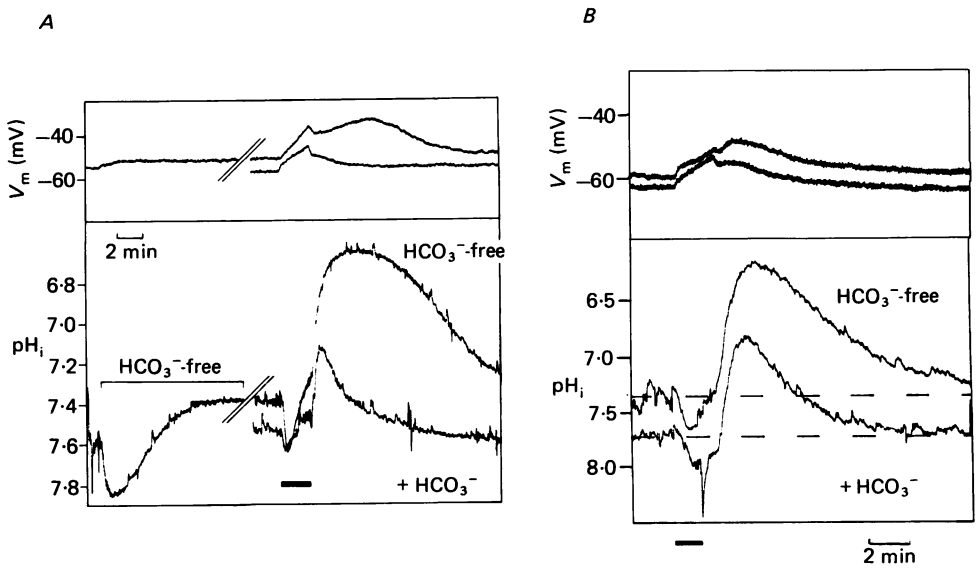

Fig. 3. Comparison of resting pH_i and pH_i recovery in HCO_3^- and *versus* HCO_3^--free solution. *A*, recovery of pH_i from acidification was determined as described in the legend to Fig. 2. The left parts of the traces show the transition from 26 mM-HCO_3^- to HCO_3^--free solution. In HCO_3^--free solution, the membrane is depolarized and the resting pH_i was more acid after a transient alkalization. The right part of the traces are superimposed recordings of membrane potential (V_m) and pH_i in HCO_3^--free solution (upper trace in each panel) and 26 mM-HCO_3^- (lower trace in each panel). After removal of NH_4^+ (application indicated by bar) pH_i recovery in HCO_3^- was rapid compared to recovery in HCO_3^--free solution. *B*, superimposed recordings of membrane potential and pH_i in HCO_3^--free solution and 26 mM-HCO_3^- obtained from a different oligodendrocyte. The differences in pH_i recovery were not as pronounced as in *A*, but still clearly visible.

of 20 mM-NH_4^+, the membrane potential first transiently, then continuously, depolarized. The pH_i increased rapidly at first then slowly returned towards the resting level. After removal of NH_4^+ membrane potential recovered and there was a rapid transient decrease in pH_i (Fig. 2). This rapid acidification varied from cell to cell and also depended on the duration of the NH_4^+ application. It typically ranged from 0·3 to 1·5 pH units. Subsequently pH_i recovered within 3–7 min (mean time until 90% of pH_i recovery was 4 min, $n = 12$) in 26 mM-HCO_3^- and within 5–11 min (mean 7 min, $n = 8$) in HCO_3^--free solution. The maximal rate of pH_i recovery could be determined by differentiating the voltage signal proportional to pH measured by the ion-selective microelectrode (Fig. 2). The mean rate of pH_i recovery was 0·2 units/min in HCO_3^--containing (range 1–0·1) and 0·1 pH units/min in HCO_3^--free

solution. The pH_i changes after application of NH_4^+ could be repeated many times on one oligodendrocyte and the steady-state pH_i was constant for many hours (in one fortunate case up to 14 h). Application and removal of NH_4^+ therefore proved to be a valuable method for studying pH_i recovery in oligodendrocytes.

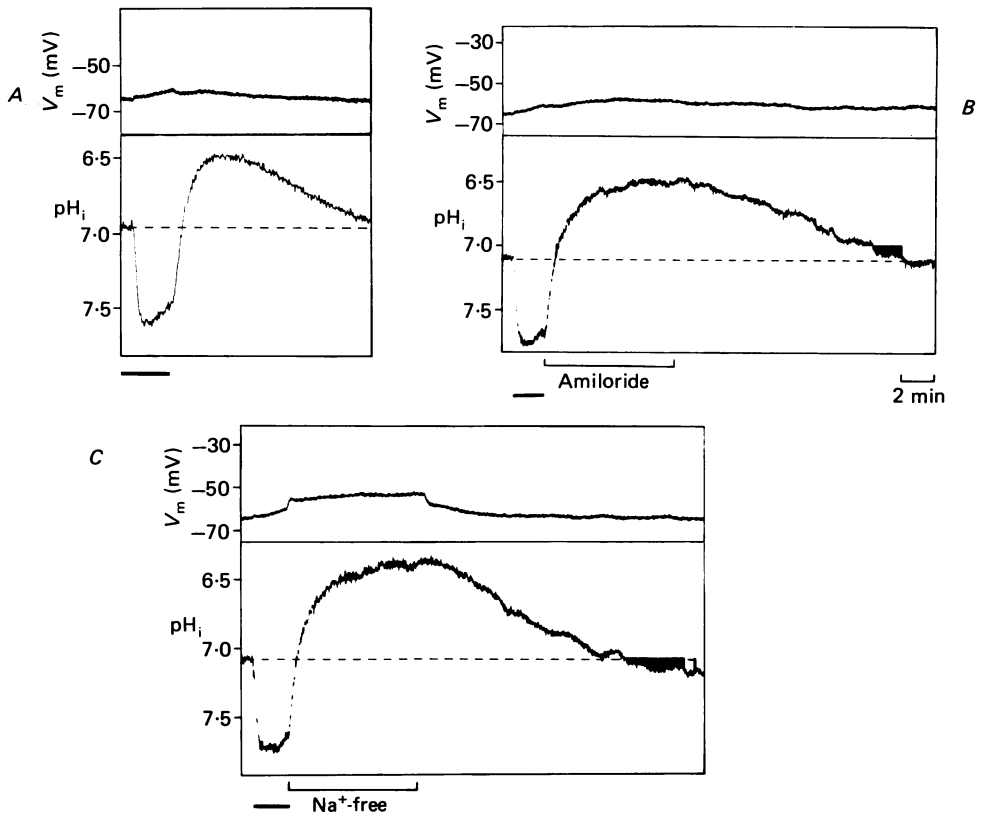

Fig. 4. pH_i recovery in HCO_3^--free solution: effect of Na^+-free solution and amiloride application. In HCO_3^--free solution pH_i recovery was determined as described in the legend to Fig. 2. The unlabelled, thick bar denotes NH_4^+ application. In Na^+-free solution (substituted by choline) pH_i recovery was blocked (*B*) in comparison to a control (*A*). Addition of Na^+ resulted in a subsequent recovery of pH_i. Similarly addition of amiloride (1 mM, *C*) blocked pH_i recovery and removal of amiloride led to a subsequent recovery.

HCO_3^--free solutions and pH_i recovery

Recovery of pH_i was compared in HCO_3^--containing and HCO_3^--free solutions to differentiate between HCO_3^--dependent and -independent pH_i-regulating mechanisms. The rate of pH_i recovery was significantly slowed in nominally HCO_3^--free salines and, as described above, the steady-state pH_i was lower ($n = 4$). The difference in the pH_i recovery in HCO_3^--containing solution as compared to a HCO_3^--free medium varied from cell to cell as illustrated in Fig. 3.

To study HCO_3^--independent carriers pH_i recovery was investigated in nominally

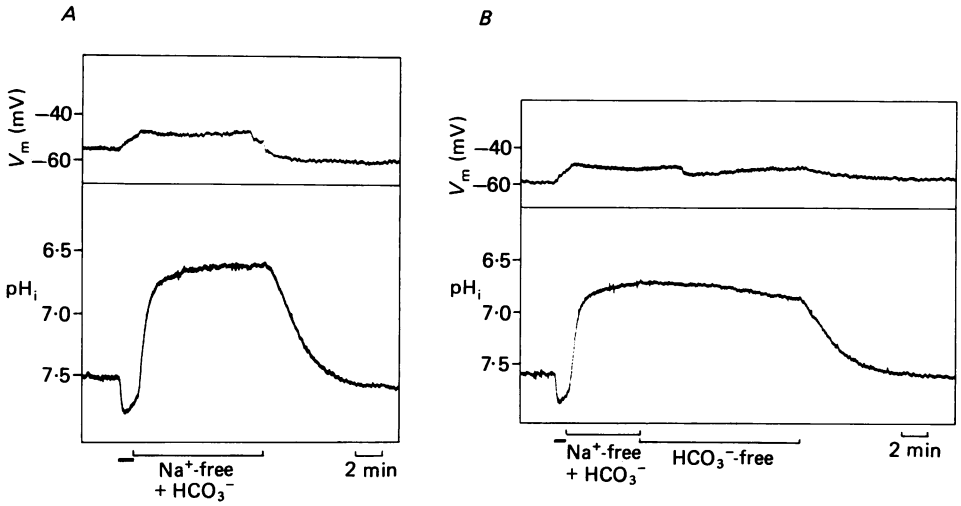

Fig. 5. The pH_i recovery in the presence of HCO_3^-: effect of Na^+-free solution. *A*, an oligodendrocyte was acidified as described in the legend to Fig. 2. After removal of NH_4^+ (application indicated by thick bar), Na^+-free solution with HCO_3^- was applied (indicated by thin bar). The pH_i recovery was blocked. After addition of Na^+, pH_i recovered. *B*, a similar experiment was performed as in *A*, except that Na^+ was readded in the absence of HCO_3^- (HCO_3^--free) as indicated by the bar. It led to a slow recovery of pH_i. Subsequently HCO_3^- (also in the presence of Na^+) was re-added as indicated. pH_i recovery was accelerated.

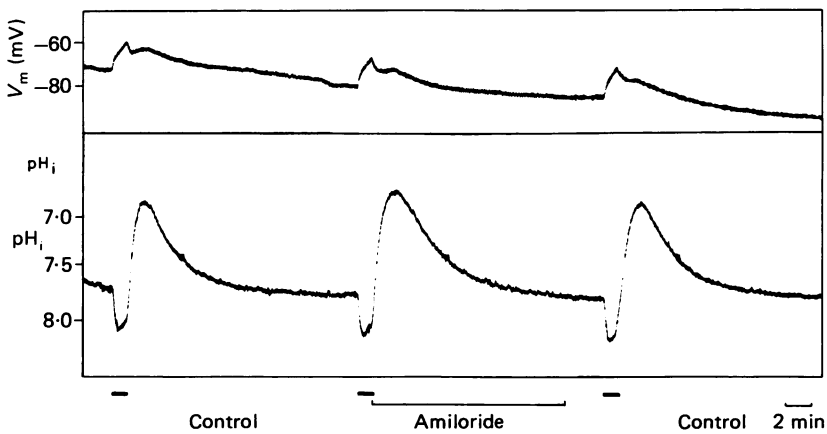

Fig. 6. Effect of amiloride on pH_i recovery in the presence of HCO_3^-. Recovery of pH_i from acidification was determined as described in the legend to Fig. 2. The unlabelled, thick bar denotes NH_4^+ application. pH_i recovery in the presence of amiloride (1 mM, application indicated by bar) was compared to controls. The rate of pH_i recovery was slightly decreased in the presence of amiloride.

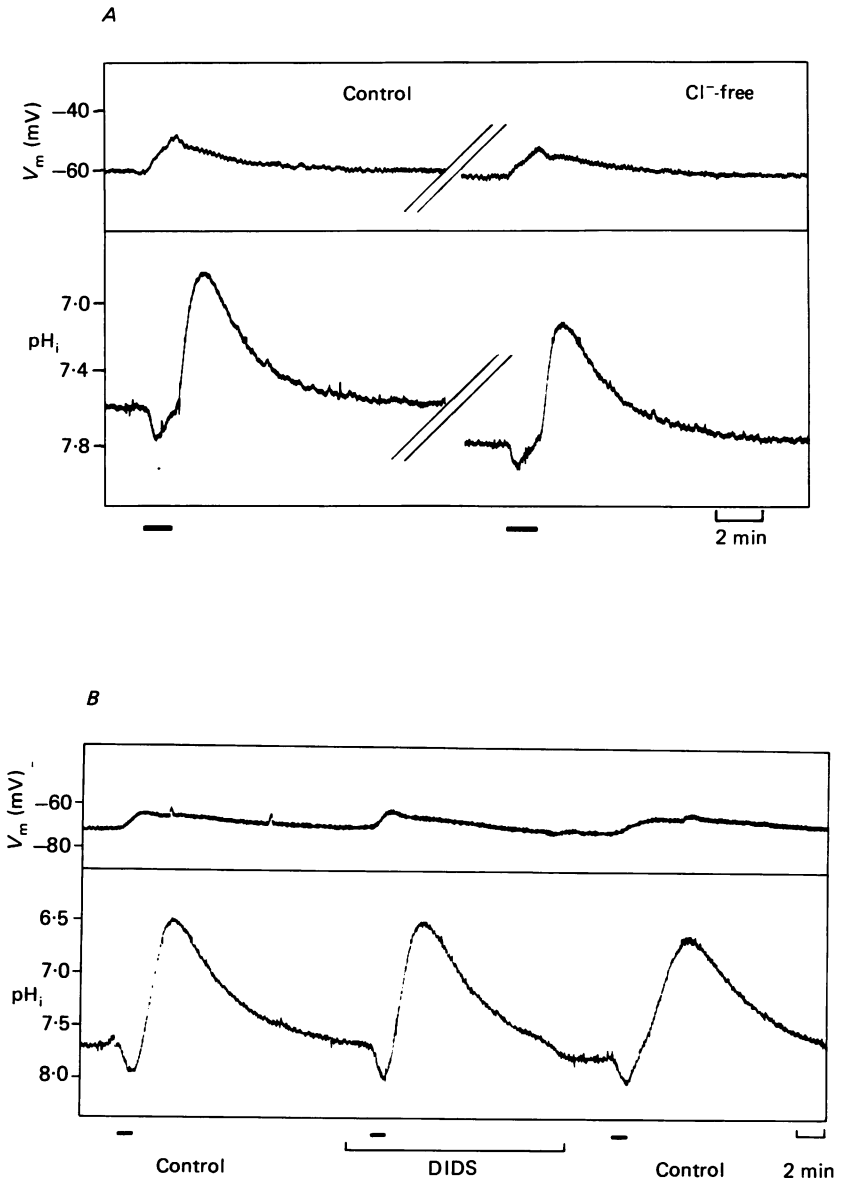

Fig. 7. Effects of $[Cl^-]_o$ and DIDS on pH_i regulation. *A*, recovery of pH_i from acidification was determined as described in the legend to Fig. 1. The unlabelled, thick bar denotes NH_4^+ application. After exposure of an oligodendrocyte to Cl^--free solution (substituted against isethionate) for 30 min pH_i recovery (Cl^--free) was compared to a control. The recovery is unaffected. Note the difference in resting pH_i. *B*, pH_i recovery in the presence of DIDS (1 mM, application indicated by bar) was compared to controls. The rate of pH_i recovery is not affected by DIDS.

HCO_3^--free solution. The pH_i recovery could be completely blocked when all extracellular Na^+ was replaced by choline or *N*-methyl-D-glucamine ($n = 7$; Fig. 4). The pH_i slowly approached the value for the passive distribution of $[H^+]$ (about 6·1). After addition of external Na^+, pH_i again recovered. Amiloride (2 mM), which has been described as specifically inhibiting Na^+–H^+ exchange (Roos & Boron, 1981), blocked pH_i recovery and wash-out of amiloride resulted in a complete restoration of the previous pH_i ($n = 3$; Fig. 4). We conclude that in the absence of HCO_3^- the pH_i regulation is accomplished by the activity of a Na^+- and amiloride-dependent mechanism.

The pH_i recovery in HCO_3^--containing solution: effect of Na^+-free solution and amiloride

The HCO_3^--mediated transport processes can be linked to Na^+, Cl^- or a combination of both (Roos & Boron, 1981; Thomas, 1984). To test the Na^+ dependence of the HCO_3^--coupled transport, pH_i recovery was investigated in Na^+-free, HCO_3^--containing solution. Figure 5 shows an experiment in which pH_i recovery was completely blocked in Na^+-free solution. The pH_i recovered after Na^+ was added to the bathing solution ($n = 10$; Fig. 5). When a Na^+-containing but HCO_3^--free solution was added after maintenance in Na^+-free solution, pH_i slowly recovered. The rate of pH_i recovery was enhanced when HCO_3^- was re-added. The addition of amiloride during pH_i recovery in HCO_3^--containing solution slightly decreased the rate of pH_i recovery, but clearly did not block it ($n = 2$; Fig. 6). These observations provide evidence that HCO_3^--coupled pH_i regulation is mediated by a Na^+-dependent, amiloride-insensitive mechanism in oligodendrocytes.

The pH_i recovery in HCO_3^--containing solution: effect of Cl^--free solution and blockers of Cl^--mediated transport processes

The HCO_3^--mediated carriers for regulation of pH_i are commonly linked to Cl^- (Roos & Boron 1981; Thomas 1984). We therefore compared pH_i recovery in Cl^--free solution with a previous control. When the bathing chamber was perfused with Cl^--free saline, large junction potentials at the reference electrode occurred. Therefore, the membrane potential given in Fig. 7*A* is corrected for these junction potentials. The resting pH_i in Cl^--free solution was more alkaline than in the control solution. This could be caused by a partial replacement of internal Cl^- by HCO_3^-. Cells were maintained in Cl^--free solution for about $\frac{1}{2}$ h and when pH_i recovery was compared to a previous control, no change in the rate of recovery was observed ($n = 2$; Fig. 7*A*). Similarly the application of Cl^-–HCO_3^- exchange blockers SITS ($n = 9$) and DIDS ($n = 4$; Fig. 7*B*) did not affect pH_i recovery. Furosemide, a blocker of Cl^- carriers, did not affect pH_i recovery either ($n = 3$; Fig. 8). However, it effectively decreased the size of the acidic shift after removal of NH_4^+, but did not slow pH_i recovery under these conditions either (Fig. 8). These results show that the HCO_3^--mediated pH_i regulation is not linked to Cl^- in cultured oligodendrocytes.

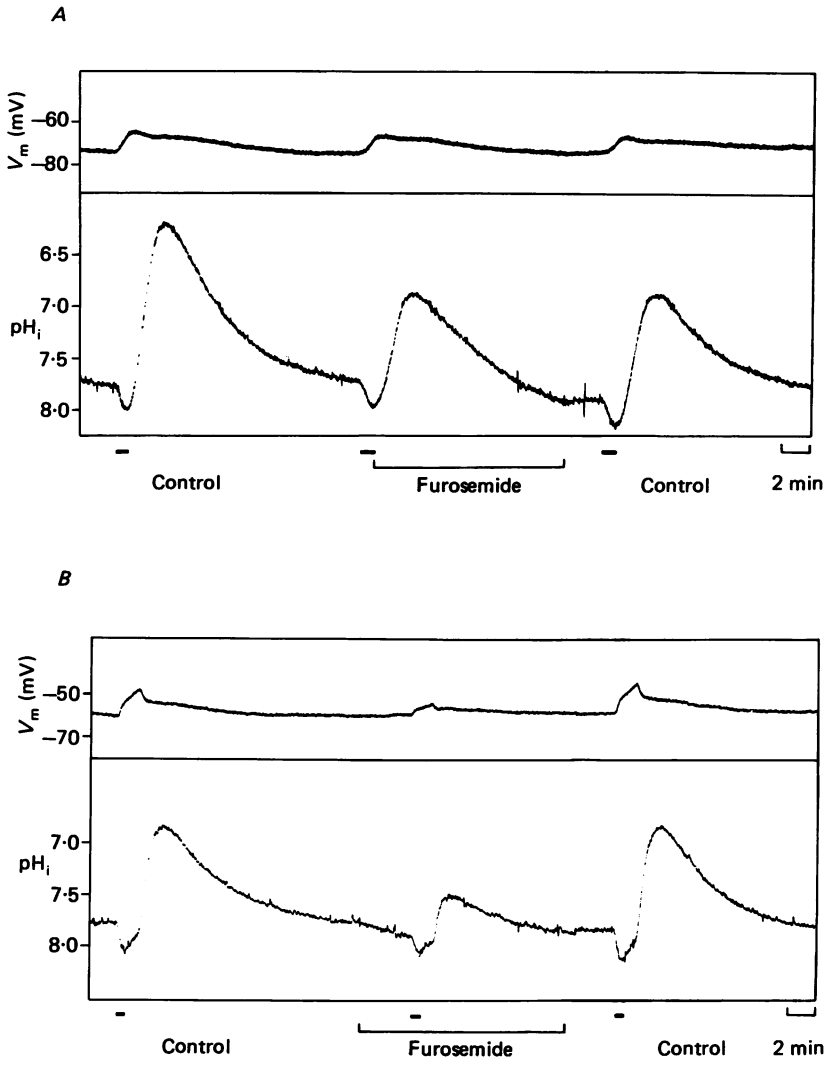

Fig. 8. Effect of furosemide on pH_i regulation. *A*, an oligodendrocyte was acidified as described in the legend to Fig. 2. The unlabelled, thick bar denotes NH_4^+ application. pH_i recovery in the presence of furosemide (1 mM, application indicated by bar) was compared to controls (Control). *B*, NH_4^+ acidification and pH_i recovery in the presence of furosemide (mM, application indicated by bar).

DISCUSSION

Steady-state pH_i

In cultured oligodendrocytes from mouse spinal cord, as in other cells studied so far (for references see Roos & Boron, 1981; Boron, 1983; Moody, 1984; Thomas, 1984), the pH_i is maintained at values too high to be explained by passive ion movements. The H^+ distribution across the glial membrane was not in equilibrium,

and an alkaline pH_i had to be maintained against an electrochemical gradient for H^+ ions. As in lamprey neurones (Chesler, 1986) the pH_i is more alkaline than the pH of the surrounding bathing medium. The pH_i values reported here, however, are different from the only other values which have been reported so far for glial cells (Deitmer & Schlue, 1987); in the medicinal leech, pH_i of glial cells is more acid (6·9–7·2) than in oligodendrocytes. The pH_i in invertebrate neurones is also commonly more acid (0·1–0·3 pH units) than the extracellular environment (Moody, 1981; Thomas, 1984; Deitmer & Schlue, 1987). It could thus be a property of cells in the vertebrate nervous system to maintain the pH_i at a more alkaline level than the pH of the external environment.

Mechanisms of pH_i regulation

Active pH_i regulation was studied by inducing an intracellular acidification by addition and subsequent removal of NH_4Cl, and observing the recovery from this acidification to normal. For recovery from acidification, there appear to be two separate mechanisms.

In nominally HCO_3^--free solution pH_i recovery from acidification is inhibited completely by removing external Na^+ or by applying 2 mM-amiloride. Both treatments are rapidly reversible. Thus pH_i recovery is mediated by a Na^+–H^+ exchanger in the glial cell membrane, as seen in many vertebrate preparations (for review see Grinstein & Rothstein, 1986) and also in crayfish (Moody, 1981) and leech (Schlue & Thomas, 1985) neurones. The existence of a Na^+–H^+ exchanger in the membrane of cultured primary astrocytes has been inferred from the observation that addition of NaCl to cells which were detached and suspended in Na^+-free medium resulted in an immediate increase in the rate of acidification (Kimelberg *et al.* 1979). The results seemed to be consistent with increased H^+ efflux due to exchange with external Na^+, but did not exclude the possibility that the acidification could also be due to increased lactate production and efflux of undissociated lactate.

However, under more physiological conditions, in the presence of HCO_3^-, our results are more complicated than with HCO_3^--free solutions. It is clear that HCO_3^- greatly stimulated the rate of acid efflux, suggesting a HCO_3^--sensitive component of normal pH_i regulation. This second pH_i-regulating mechanism of oligodendrocytes seems to be Na^+-dependent, because pH_i recovery is inhibited completely in Na^+-free solutions in the presence of HCO_3^-. In all other preparations studied such HCO_3^--dependent acid extrusion mechanisms are blocked by SITS or DIDS, but these stilbene derivatives were without effect on pH_i recovery of oligodendrocytes. The mechanism was also unaffected by removing external Cl^- (thereby depleting internal Cl^-; H. Kettenmann, unpublished observation). These observations strongly suggest that a Na^+-dependent mechanism is involved in pH_i homeostasis of cultured oligodendrocytes, which mediates the influx of HCO_3^- ions into the cell interior. The tight coupling of HCO_3^- transport to Na^+ has been reported for a variety of different animal cells (for review see Boron, 1983; Thomas, 1984). A Na^+–H^+–HCO_3^-–Cl^- exchanger was first proposed to explain acid extrusion by snail neurones (Thomas, 1977), and has subsequently been found in several other invertebrate preparations (e.g. barnacle muscle fibres, Boron, McCormick & Roos, 1981; squid axon, Boron &

Russel, 1983; crayfish central neurones, Moody, 1981; crayfish stretch receptor sensory neurone, Moser, 1985). This acid extrusion system is thought to be electroneutral because pH_i recovery occurs with no change in E_m and changes in the latter do not affect the rate of pH_i recovery (Thomas, 1978). The system is not only inhibited by removing external Na^+ or HCO_3^-, but also, in contrast to the pH_i-

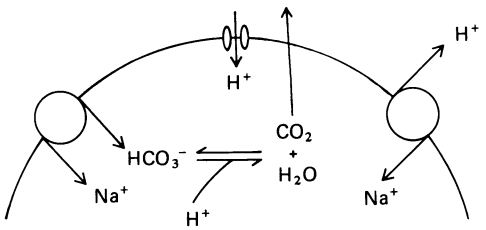

Fig. 9. Scheme of pH regulation in oligodendrocytes.

regulating mechanism in oligodendrocytes, by applying SITS or DIDS or by depleting internal Cl^- (Thomas, 1977). A symport of Na^+ and HCO_3^-, which is also inhibited by SITS or DIDS, has been suggested to operate in a variety of cells involved in transepithelial transport of HCO_3^- ions (Boron & Boulpaep, 1983; Jentsch, Keller, Koch & Wiederholt, 1984; Jentsch, Stahlknecht, Hollwede, Fischer, Keller & Wiederholt, 1985*a*; Jentsch, Schill, Schwartz, Matthes, Keller & Wiederholt, 1985*b*; Alpern, 1985; Yoshitomi, Burckhardt & Frömter, 1985; Biagi & Sohtell, 1986). This co-transporter is electrogenic, and thought to tightly couple the movement of one Na^+ ion to the transport of two (Boron & Boulpaep, 1983) or three (Yoshitomi *et al.* 1985) HCO_3^- ions, thus leading to a transfer of net negative charge.

We conclude that in HCO_3^--containing media pH_i regulation by cultured oligodendrocytes from mouse spinal cord has at least two mechanisms, one being Na^+–H^+ exchange and the other Na^+- and HCO_3^--dependent. It is most likely that a stilbene-insensitive Na^+–HCO_3^- co-transport could be responsible for an inward transport of HCO_3^-, but the stoichiometry of this mechanism is unknown (Fig. 9).

Functional consequences and comparison to astrocytes

In the extracellular environment of the intact nervous system $[HCO_3^-]$ is about 15–20 mM and thus similar to the activity in the bathing solution. The HCO_3^--dependent mechanism will thus be the dominant mechanism regulating pH_i in oligodendrocytes of the intact nervous system assuming that they have similar properties to oligodendrocytes in culture. The exclusive dependence of pH_i regulation in oligodendrocytes on the Na^+ gradient and its independence from the Cl^- gradient suggests an entirely independent $[Cl^-]_i$ regulation. Since astrocytes, unlike oligodendrocytes, have been shown to possess a Cl^-–HCO_3^- exchanger, alteration in cell Cl^- is likely to affect intracellular astrocytic pH by affecting HCO_3^- levels (Kimelberg *et al.* 1979; Kimelberg, 1981; Lowe & Lambert, 1983). Both

oligodendrocytes and astrocytes actively accumulate Cl^-, but to a different extent (Kimelberg, 1981; Kettenmann, 1987). These two glial subpopulations are therefore different in their expression of Cl^- carriers, one linked to the other independent of pH regulation. Astrocytes and oligodendrocytes possess a GABA-activated Cl^- conductance causing a depolarization of the membrane potential upon activation (Gilbert, Kettenmann & Schachner, 1984; Kettenmann, Backus & Schachner, 1987). Since, in contrast to astrocytes, $[Cl^-]_i$ and pH_i are independently controlled in oligodendrocytes, activation of GABA receptors could alter pH only in astrocytes, but not in oligodendrocytes.

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